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Dalbavancin Disk Diffusion Tests: Development Evaluations Using Various Disk Concentrations, Solvents and Surfactants

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ABSTRACT

Background:

Poor agar diffusion of high molecular weight antimicrobials compromise susceptibility (S) testing by CLSI disk diffusion (DD) method. Examples of suboptimal performance have led to CLSI DD interpretive disclaimers for polymyxins, vancomycin and daptomycin. Generally, organisms having elevated MICs (resistant [R]) may have DD zones indistinguishable from wildtype (WT) S strains. Dalbavancin (DAL), a long-acting lipoglycopeptide, was evaluated for possible DD testing at two reference sites.

Methods:

Complementary studies at JMI (J) and Eurofins (E) were performed by the CLSI M2-A9 method as follows: disk ranges (5, 10, 15, 30-µg [E] or 15, 30, 60-µg [J]); DAL ± DMSO at 12 or 100% (E) and 100% ± polysorbate-80 (P-80; J); and 15 or 30-µg DAL disks + 0.001 to 1% P-80 ± DMSO (J). Single or replicate tests of WT or R subsets were tested using 30-µg DAL disks versus: WT-MSSA including QC strains (12), WT-MRSA (20), VISA (13), VRSA (5), VRE (17), WT-enterococci including ATCC 29212 (5), *S. epidermidis* (SEPI; 20), ß-streptococci (21) and pneumococci (11). Vancomycin 30-µg disks were used as controls.

Results:

Disk conc. studies demonstrated WT strain zone diameters were not significantly increased at >30-µg. Inhibitory zones (>6 mm) were not observed around disks charged with DMSO or P-80 (100 and 1%, respectively). Addition of DMSO and P-80 did not significantly enhance DAL 30-µg DD zones. 30-µg DAL disk zones for WT-S strains (range in mm) were: MSSA or MRSA (14-19), SEPI (15-19) and streptococci (14-20); and glycopeptide non-S strains ranged: VRE (6-21), *vanC* enterococci (8-16), VRSA (6-14) and VISA (13-17). WT strains with DAL MICs at ≤0.12 µg/ml had 30-µg DAL DD zones at ≥14 mm, but discrimination from strains with DAL MIC, 0.25-4 µg/ml (VISA, *vanA* or *C* enterococci, VRSA) was poor. All QC strains (4) had DAL 30-µg DD zones of 14-18 mm.

	Zone DAL disk conc. (µg)[a]			DMSO at 100%[a]	
Organism	15	30	60	Alone	+P-80
MSSA ATCC 25923	16.8	16.8	16.8	6.0	6.0
MSSA ATCC 29213	15.9	16.3	16.8	6.0	6.0
E. faecalis ATCC 29212	15.8	16.4	16.4	6.0	6.0
WT-*S. pyogenes*	16.4	16.5	16.8	6.0	6.0
S. pneumoniae ATCC 49619	17.5	17.3	17.0	6.0	6.0

a. Average diameter in mm of 12 observations.

Conclusions:

DAL DD tests, like other heavy peptide agents, produce small zones of inhibition with poor discrimination between WT-S strains (zones, ≥14 mm) and those with higher DAL MIC values. DAL S should optimally be tested by the CLSI broth microdilution method or by a validated commercial method (Etest).

INTRODUCTION

Dalbavancin is a "second-generation" lipoglycopeptide, synthesized by dimethylaminopropyl amide derivatization of the natural glycopeptide A40926 which has completed phase III clinical trials in skin and skin structure infections. Results of Phase II studies in skin and skin structure infections and catheter-related bacteremia have been published. Dalbavancin has exhibited a wide spectrum of activity against Gram-positive bacteria, including methicillin-resistant staphylococci and other multidrug-resistant organisms. Dalbavancin's mechanism of action consists of inhibiting cell-wall peptidoglycan cross-linking, similar to that of other glycopeptides, but the agent is not completely cross-resistant to vancomycin. It has been shown to be more potent than vancomycin and teicoplanin and is active against *vanB* vancomycin-resistant enterococcal (VRE) strains. The extended half-life of dalbavancin (approximately 8 days) permits once-weekly dosing, making it an advantageous therapeutic option compared to similar acting glycopeptides.

This new lipoglycopeptide has been shown to be bactericidal without a propensity towards the development of resistance during in vitro serial passaging and direct selection studies. Extensive pharmacokinetic, pharmacodynamic and Monte Carlo simulation studies suggest potential susceptible breakpoint concentrations ranging from ≤0.5 µg/ml (staphylococci) to ≤4 µg/ml (streptococci; Pfizer Inc., unpublished data). Candidate dalbavancin interpretative criteria for MICs of ≤0.5 and ≤1 µg/ml have been used in prior analyses to determine if currently tested glycopeptides in commercial systems (validated against reference test) could predict dalbavancin susceptibility with acceptable accuracy. The surrogate marker agents (vancomycin or teicoplanin) were observed to be highly predictive and produced only minor error (false-intermediate) when using the CLSI vancomycin breakpoint.

Poor diffusion in agar of high molecular weight antimicrobials (like dalbavancin) compromises susceptibility testing by the commonly used CLSI disk diffusion method. Examples of suboptimal performance have led to disk diffusion test interpretive disclaimers in recent years for polymyxins, vancomycin and daptomycin. In this study, we assess the possibility of developing a simple and predictive dalbavancin disk diffusion test that can be immediately available post US-FDA approval. Dry-form broth microdilution trays (TREK Diagnostics, Cleveland, OH) and Etests (AB BIODISK, Solna, Sweden) have been previously validated for diagnostic use, correlating well to reference CLSI MIC values.

MATERIALS AND METHODS

Complimentary investigations of the dalbavancin disk diffusion method were performed by JMI Laboratories (North Liberty, Iowa) and Eurofins Medinet (Herndon, Virginia). Each group performed the broth microdilution and disk diffusion tests by Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) methods as published in documents M2-A9 (2006) and M7-A7 (2006). Quality control and interpretation of breakpoints for the internal control (vancomycin) were derived from CLSI M100-S17 (2007).

Numerous candidate dalbavancin disk concentrations were tested (5-, 10-, 15-, 30-, 60-µg) each prepared by the investigators. DMSO solvent was used in varying concentrations (1-100%) and the surfactant recommended for use by the CLSI in the dalbavancin reference broth microdilution test, polysorbate-80, was also applied to disks in concentrations ranging from 0.001 to 1% (0.02% for the MIC method). Combinations of DMSO, polysorbate-80 and dalbavancin were also investigated.

Multiple strains of Gram-positive species were tested including:

- Quality control organisms (see Tables 1-3; four strains)
- Wildtype (WT) methicillin-susceptible *S. aureus* (MSSA; 10 strains)
- WT methicillin-resistant *S. aureus* (MRSA; 20 strains)
- Vancomycin-interemediate *S. aureus* (VISA;13 strains)
- Vancomycin-resistant *S. aureus* (VRSA; 5 strains)
- Vancomycin-resistant enterococci (VRE; 17 strains)
- WT enterococci (2 species; 4 strains)
- *S. epidermidis* (20 strains)
- ß-haemolytic streptococci (21 strains)
- *S. pneumoniae* (10 strains)

RESULTS

- Dalbavancin is a large lipoglycopeptide molecule that has a long serum elimination half-life and diffuses poorly through an agar matrix. Previous experience with the broth microdilution test demonstrates significant dalbavancin binding to the plastic surfaces of microtitre plates requiring the use of low concentrations of a surfactant, polysorbate-80 (P-80; 0.002%) in frozen MIC panels.

- Tests to select an optimal dalbavancin disk diffusion concentration showed the following:
  - Small zone diameters for susceptible wildtype (WT) Gram-positive strains, generally ≤15 mm.
  - Zones increased in size slightly (3-4 mm) over the disk content range of 5- to 30-µg, but <1 mm overall when increased from 30- to 60-µg (Tables 1 and 2).

- Potential use of high solvent (DMSO) and/or surfactant (P-80) concentration alone had no direct antimicrobial effects on Gram-positive pathogens (Table 2), and dalbavancin zones generally were 2-5 mm smaller than vancomycin when using the same 30-µg disk concentration.

- Replicate tests with WT or quality control strains using 30-µg dalbavancin disks with DMSO solvent (≥1%) and varying amounts of P-80 did not improve zone diameters (average, +1.4 mm, range NC [no change] to 2.2 mm; see Table 3).

Table 1. Solvent (DMSO) and dalbavancin disk concentration studies using three *S. aureus* strains tested in triplicate (Eurofins Medinet).

		Zone diameter for *S. aureus* in mm[a]		
Solvent concentration (%)	Disk concentration (µg)	ATCC 25923 (WT)	1079896 (VISA)	1077924 (VRSA)
12	5	11.7	11.7	6.0
	10	13.3	14.7	6.0
	15	14.0	16.0	6.0
	30	15.3	16.7	6.0
100	5	12.3	13.0	6.0
	10	14.0	15.7	6.0
	15	15.3	15.7	6.0
	30	16.0	17.0	6.0

a. Average of triplicate tests. Vancomycin 30-µg disk controls were 6.0-20.0 mm. WT = wildtype.

- Testing with a large population (113 strains) of dalbavancin-susceptible and -resistant organisms (Table 4), the disk method demonstrated suboptimal performance:
  - Poor discrimination of strains with dalbavancin MIC results over the range of 0.25-4 µg/ml (zones 12-14 mm).
  - Most WT strains had zones of ≥14 mm, however, the smallest zone (14 mm for strains with dalbavancin MICs ranging from ≤0.03-0.5 µg/ml) could not be distinguished from documented VRSA, VRE and VISA isolates.

Table 2. Dalbavancin disk diffusion test zone diameters for three drug concentrations including solvent, surfactant and vancomycin controls (JMI Laboratories).

	Mean zone diameter (mm):[a]					
	Dalbavancin disk					
Organism	15-µg	30-µg	60-µg	DMSO	DMSO + P-80[b]	Vancomycin
S. aureus ATCC 25923	16.8	16.8	16.8	6.0	6.0	18.7
S. aureus ATCC 29213	15.9	16.3	16.8	6.0	6.0	17.3
E. faecalis ATCC 29212	15.8	16.4	16.4	6.0	6.0	17.2
S. pyogenes ATCC 19615	16.4	16.5	17.8	6.0	6.0	21.7
S. pneumoniae ATCC 49619	17.5	17.3	17.0	6.0	6.0	21.0

a. Average of 12 observations for dalbavancin, and six results for the vancomycin control.
b. P-80 = Polysorbate-80 surfactant.

Table 3. Average of six replicate zone diameters testing eight selected Gram-positive organisms with 30-µg dalbavancin (having nil, 0.001, 0.01, 0.1 and 1% polysorbate-80 [P-80]) and 30-µg vancomycin disks (JMI Laboratories).

		Dalbavancin zone (mm) by P-80 concentration (%):				
Organism[a]	Vancomycin zone (mm)	Nil	0.001	0.01	0.1	1 (variation)
S. aureus ATCC 25923	17.7	14.8	15.9	15.7	16.0	17.0 (+2.2)
S. aureus ATCC 29213	18.7	14.7	16.0	15.7	16.0	16.9 (+2.2)
E. faecalis ATCC 29212	18.0	15.5	15.7	16.0	17.0	16.9(+1.4)
S. pneumoniae ATCC 49619	23.2	15.7	16.5	16.1	16.7	17.0 (+1.3)
S. pyogenes[b]	22.8	15.7	16.3	16.1	16.5	17.2 (+1.5)
E. faecalis VRE	10.2	20.3	20.7	19.7	20.7	20.3 (NC)
S. aureus VRSA	12.8	13.9	14.0	12.9	13.0	13.9 (NC)
S. aureus VISA	17.7	14.8	15.3	14.9	15.0	14.9 (+0.1)

a. VRSA = vancomycin-resistant *S. aureus*, VISA = vancomycin-intermediate *S. aureus*, VRE = vancomycin-resistant enterococci (*vanB*).
b. Wildtype strain.

Table 4. Distribution of 30-µg dalbavancin disk zone diameters (mm) compared to CLSI reference MIC results (Eurofins Medinet) for 113 strains.

		Dalbavancin	Zone diameter in mm:													
Organisms[a]	Resistances[b]	MIC (µg/ml)	6	7	8	9	10	11	12	13	14	15	16	17	18	≥19
SA, ENT	VRSA, VRE (ABC)	≥32	4	-	-	1	-	-	-	-	-	-	-	-	-	-
ENT	VRE (ABC)	16	1	-	1	-	1	1	-	-	-	-	-	-	-	-
ENT	VRE (ABC)	8	1	-	2	-	-	-	-	-	-	-	-	-	-	-
SA, ENT	VRSA, VRE (AC)	4	-	-	-	-	1	-	2	-	1	-	-	-	-	-
SA	VISA	2	-	-	-	-	-	-	-	1	-	1	-	-	-	-
SA	VISA	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-
SA	VISA	0.5	-	-	-	-	-	-	-	-	1	3	-	-	-	-
SA, ENT	VISA, VRE (B)	0.25	-	-	-	-	-	-	-	-	1	2	-	-	-	-
SA, ENT	VISA, VRE (C)	0.12	-	-	-	-	-	-	-	-	-	3	3	-	-	-
SA, ENT, ST	-	0.06	-	-	-	-	-	-	-	-	2	30	5	2	-	-
SA, ENT, ST	-	≤0.03	-	-	-	-	-	-	-	-	7	9	3	11	6	5

a. SA = *S. aureus*, ENT = enterococci, ST = streptococci (*S. pneumoniae* and ß-haemolytic species).
b. VRSA = vancomycin-resistant SA, VRE = vancomycin-resistant enterococci, VISA = vancomycin-intermediate SA, (ABC) = vanA or B or C resistance pattern.

- Control vancomycin disk test results had similar issues of poor performance (data not shown) in this experiment validating recent warnings by the CLSI of possible testing error when using this method.

CONCLUSIONS

- Dalbavancin disk diffusion testing has inherent technical problems in common with other large peptide molecules (polymyxins, lipopeptides, glycopeptides).

- Additive solvents and/or surfactants at low or high concentrations do not improve dalbavancin disk diffusion test performance.

- Laboratories needing to measure dalbavancin in vitro susceptibility should utilize validated dilution tests such as reference-quality broth microdilution methods with 0.002% P-80, surrogate marker testing (vancomycin or teicoplanin) or Etest (AB BIODISK, Solna, Sweden). The latter method will require care/experience for interpretation of the endpoint due to narrow ellipses or fuzzy zone borders.

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